Development of a New in vivo Double Autoradiogram for the Analysis of Dopaminergic System of the Rat Brain

III. 5. Development of a New in vivo Double Autoradiogram for the Analysis of Dopaminergic System of the Rat Brain

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Introduction

It is well known that neurotransmitters released from the nerve terminals can interact with their specific receptors on the postsynaptic membrane in order to send signals and that such responses of the signal transducing systems carried out at the synapses play quite important roles in brain function. In the present study, we employed double-labeled autoradiography in an attempt to simultaneously observe the physiological conditions *in vivo* of both the neurotransmitter, dopamine, at the presynaptic sites and the binding activities of the specific receptor, dopamine D_2, at the postsynaptic sites of the striatum of the rat brain which were distributed in the dopaminergic nerve terminals of the nigrostriatal tract. A tritiated form of YM-09151-2,cis-N-(1-benzyl-2-methyl-pyrrolidin-3-yl)-5-chloro-2-methoxy-4-methyl-aminobenzamide, a potent dopamine D_2 receptor antagonist, has been used to label D_2 receptors in dog [1] and rat [2] striatal membrane homogenates with high affinity and low levels of non-specific binding. Dopamine and dopamine D_2 receptor binding sites were determined by using [^{18}F]-6-fluorodopa (^{18}F-DOPA) and [^{3}H]YM-09151-2, respectively.

Materials and Methods

Three adult male Wistar rats weighing 280-300 g were allowed free access to food and water. [^{18}F]-DOPA was synthesized by the method described by Adam et al. [3] at the Cyclotron Radioisotope Center of Tohoku University. A 0.7-ml physiological saline containing 0.3 mCi [^{18}F]-DOPA and 20 μCi [^{3}H]YM-09151-2 (New England Nuclear, spec. act. 86.1 Ci/mmol) was administered intravenously under anesthesia with a gas mixture of 2% halothane, 70% nitrous oxide, and oxygen. After injection, anesthesia was discontinued and the animals were allowed free access to food and water until decapitation. Forty-five minutes after injection, the animals were sacrificed and the brains were quickly removed and frozen in powdered dry ice. Serial coronal sections 20 μm in thickness were cut from the frozen brain in a -20°C cryostat and dried at 60°C on glass cover slips. [^{18}F]-DOPA autoradiograms were prepared from these sections by exposing them to the specific imaging plates for positron tracers (BAS-UR, Fuji Photo Film Co., LTD.) for 12 hours in standard

X-ray cassettes. After decay of [^{18}F] radioactivity, those sections were exposed to selective tritium sensitive imaging plates (BAS-TR, Fuji Photo Film Co., LTD.) for two weeks in order to obtain [^{3}H]YM-09151-2 autoradiograms. The autoradiograms were scanned by the bioimaging analyzer system (BAS 3000) developed by Fuji Photo Film Co., LTD., Japan.

Results

The [^{18}F] radioactivity of the adjacent section of each exposed section was measured using a gamma counter to obtain the mean exposed radioactivity of the brain section. The mean value of [^{18}F] radioactivity from the brain sections measured 90 min after the injection of 0.3 mCi of [^{18}F]-DOPA was 140 ± 55 (mean ± S.D.) counts/min.

Two different autoradiograms from the same brain sections at the level of the striatum and the substantia nigra are shown in Fig. 1. The accumulation of [^{18}F]-DOPA was concentrated at the presynaptic sites of the striatum where dopaminergic nerve terminals of the nigrostriatal tract were located (Fig. 1a). The distribution of [^{3}H]YM-01951-2 binding was found to be densest in brain structures known to be rich in dopamine D_2 receptors. [^{3}H]YM-09151-2 accumulated selectively at the postsynaptic sites of the striatum and the substantia nigra, findings which were in agreement with those previously reported for an in vitro autoradiographic method using the same ligand [4] (Fig. 1b).

Discussion

It is well known that multi-labeled autoradiographic techniques using tracers with different half-life periods can be utilized to obtain different physiological information from the same animal or specimen. For instance, in the field of brain research, that method has been utilized for the simultaneous observation of both metabolism and blood flow[5] and for observation of the sequential changes of the cerebral blood flow in the ischemic brain of the rats at different stages using the same animals.[6]

On the other hand, in the case of the investigation of neurotransmitters and receptors of the brain, there have been many reports of research in which enzyme histochemistry or radiolabeled receptor assay was employed. Using brain sections, the in vitro rather than the in vivo autoradiographic method has been more commonly utilized, because the amount of administered radioligand that was transferred to the brain was limited and there were more non-specific binding sites detectable by using the in vivo autoradiographic method than the in vitro method. Therefore, it was difficult to obtain clear imaging data with the in vivo autoradiographic method. Moreover, it is not easy to analyze in vivo autoradiographic data quantitatively since metabolism of the administered radioligand in vivo is not always simple and various factors involved in the metabolic process in vivo must be considered.

In the present study, however, we could obtain clear imaging data by using imaging plates with selective radio-sensitivity and the newly developed bioimaging analyzer system. The in vivo autoradiographic method has the marked advantage of observation under more

physiological and more natural conditions of the brain than the in vitro method. This method is quite efficacious for simultaneous observation of both the neurotransmitter system at the presynaptic sites and the receptor system at the postsynaptic sites of the same brain in vivo, and will prove to be an important tool for investigations of various brain diseases in animal models.

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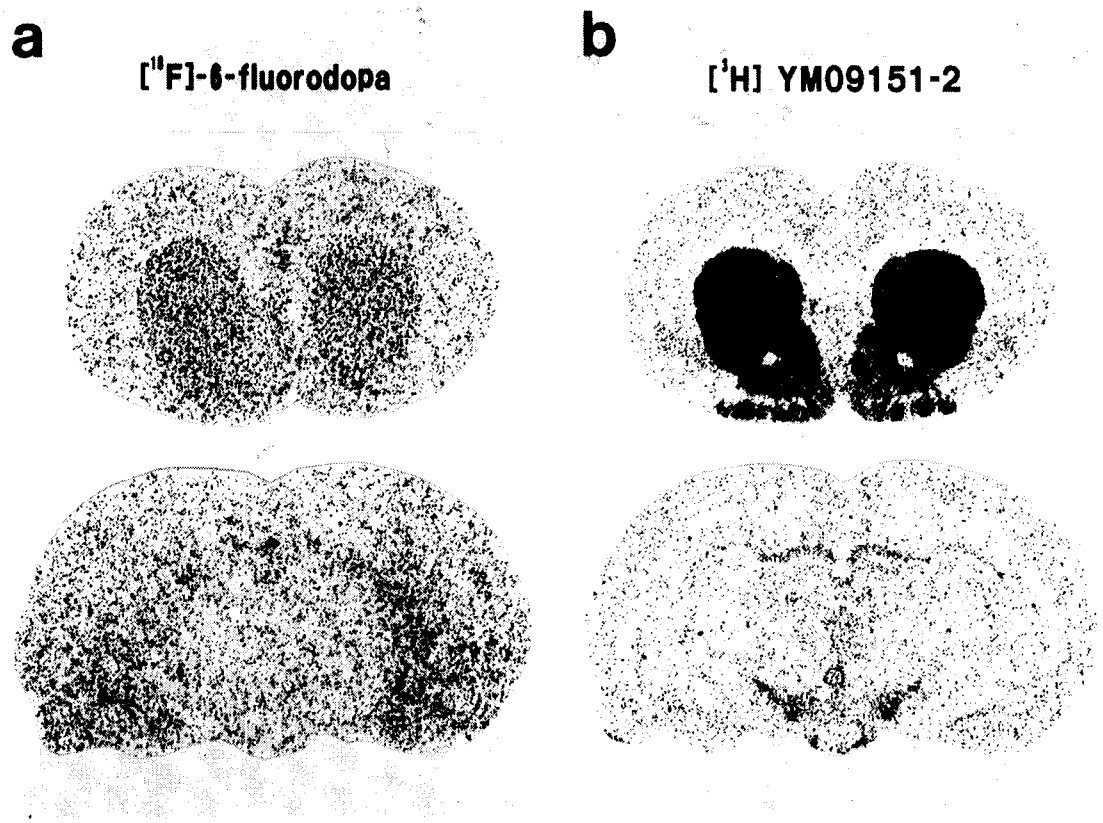

Fig. 1. [^{18}F]-L-fluoro dopa (a) and [^{3}H]-YM-09151-2 (b) autoradiograms of the same sections of the rat brain. Representative autoradiograms show coronal sections at the level of the striatum (upper) and the substantia nigra (lower).